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Review

Toxoplasmosis in pigs—The last 20 years

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ABSTRACT

Pigs are important to the economy of many countries because they are a source of food for humans. Infected pig meat is a source of *Toxoplasma gondii* infection for humans and animals in many countries. This parasite also causes mortality in pigs, especially neonatal pigs. Most pigs acquire *T. gondii* infection postnatally by ingestion of oocysts from contaminated environment or ingestion of infected tissues of animals. Few pigs become infected prenatally by transplacental transmission of the parasite. Raising pigs indoors in confinement has greatly reduced *T. gondii* infection in pigs but the recent trend of organic farming is likely to increase *T. gondii* infection in pigs. Recently, feeding goat whey to pigs was found to be a risk factor for *T. gondii* infection in organically raised pigs. Currently used molecular and histopathological methods are insensitive for the detection of *T. gondii* in pork because of the low concentration of the parasite in meat destined for human consumption. There is no vaccine to prevent *T. gondii* infection in pigs but efforts are being continued to develop a non-viable vaccine. In the present paper, information on prevalence, transmission, diagnosis, and control of porcine toxoplasmosis in the last 20 years (since 1988 when last reviewed by this author) is reviewed. Worldwide reports of clinical and asymptomatic infections in pigs are reviewed. Methods to detect *T. gondii* in pigs are compared. Recent studies on genetic typing of *T. gondii* strains prevalent in pigs are discussed with respect to epidemiology. Because wild pigs are hunted for food for human consumption prevalence in wild pigs is summarized.

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1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988; Dubey, 2009). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. The proportion of the human population that acquires infection by ingestion of oocysts in the environment or by eating contaminated meat is not known and there are currently no tests available that can determine the infection source. However, sero-epidemiologic data suggest that ingesting improperly cooked meat containing *T. gondii* is a major source of infection for humans in the USA (Dubey and Jones, 2008).

To the author's knowledge, there is no identification system for individual pigs destined for human consumption in the USA and probably other countries, and pigs are not tested for *T. gondii* infection at slaughter in any country. Therefore, the routes by which *T. gondii*–infected pigs from highly endemic areas enter the market and the role these pigs have in the overall epidemiology of *T. gondii* in humans remains unknown. We have previously reviewed worldwide information on toxoplasmosis in pigs up to 1988 (Dubey, 1986; Dubey and Beattie, 1988). In the present paper, worldwide information on prevalence, transmission, and control of toxoplasmosis in pigs since 1988 is reviewed.

2. Natural infections in domestic pigs

2.1. Serologic prevalence

Surveys based on the presence of antibodies in blood-sera have reported a worldwide distribution of *T. gondii* infection in pigs (Table 1). Little information is available concerning *T. gondii* infection in pigs in People's Republic of China (PRC) because the information is published in Chinese and in local journals. I requested Prof. Zhu to summarize seroprevalence data in pigs from PRC and this is to be published by Zou et al. (in press).

Most of these studies in Table 1 were based on convenience samples collected from slaughtered pigs. Prevalence of *T. gondii* varied dramatically among the classes of pigs surveyed (market pigs versus sows, indoor pigs from biosecure housing systems versus free-range). In the USA, the pigs used for unprocessed pork consumption (feeder pigs, market pigs, finisher pigs – these terms are synonymous) are mostly raised indoors in well managed facilities. In these well managed facilities, prevalence of *T. gondii* has declined drastically in the last decade (Table 1). In a statistically valid population-based nationwide survey (the sample size was proportional to the number of pigs slaughtered) conducted in 1983–1984, seroprevalence was 23% in market pigs and 42% in breeder pigs (sows) (Dubey et al., 1991a). When pigs from these same areas were tested in 1992, *T. gondii* seroprevalence had dropped to 20.8% in breeders and 3.1% in finisher pigs (Dubey et al., 1995a). The institution of a National Animal Health Monitoring System (NAHMS) for swine now allows periodic surveillance of pigs for microbial infections in the USA. The prevalence of *T. gondii* in four NAHMS swine surveys in 1990, 1996, 1998, and 2006 showed a steady decline in pigs in the USA (Table 1). The prevalence of *T. gondii* in pigs is also influenced by management systems. In poorly managed non-confinement systems, seroprevalence in pigs was as high as 68% (Gamble et al., 1999).

The higher seroprevalence in sows compared with market age pigs is epidemiologically relevant with respect to transmission of *T. gondii*; market age pigs are sold for use in fresh, unprocessed pork products whereas meat from breeding sows is usually processed (such as sausages, salami, etc.) and processing kills or reduces *T. gondii* in pork. In the 1990s seroprevalences also decreased in pigs under intensive managements in some European countries. For example, in Austria, seroprevalence of 14% in 1982 decreased to 0.9% in 1992 (Edelhofer, 1994). The recent trend of rearing pigs outdoors in European countries is likely to increase seroprevalence in pigs in The Netherlands (Meerburg et al., 2006; van der Giessen et al., 2007; Kijlstra et al., 2004, 2008. The definition for organic and free-range (FR) pigs provided by Kijlstra et al. (2004) is repeated here. There are three classifications of pigs in The Netherlands. "Finishing pigs" are housed indoors, usually on concrete floors, are fed regular pig feed, and come from intensive farms. FR pigs, on the other hand, are allowed access to outside enclosures and have straw bedding; they too are fed regular pig feed. Organic pig raising is controlled by a set of regulations crafted by the European Union (EU regulation 2092/91), which stipulate access to the outside, straw bedding, and organic pig feed, which often contains the same plant ingredients as regular pig feed, but is grown on farms that do not use artificial chemical fertilizers or pesticides. Other differences on organic pig farms include greater living space provided for organic pigs than on intensive farms, clipping teeth and cutting tails are prohibited, animals are weaned at a later age, and use of antibiotics and drugs is restricted. The waiting time after use of antibiotic or drug treatments is twice that of regular pig farms. Organic slaughter pigs are allowed only one synthetic drug or

Table 1
Seroprevalence of *T. gondii* antibodies in domestic pigs.

Country	Reference	Test	No. exam	%Pos.	Cut-off titer	Notes
Argentina	Omata et al. (1994)	IFA	109*	63.3	64	b
	Venturini et al. (2004)	MAT	230	37.8	25	a,b
Austria	Edelhofer (1994)	IFA	*2238 (1982)	12.2	16	b
		IFA	†113 (1982)	43.4	16	b
		IFA	*2300 (1992)	0.8	16	b
		IFA	†46 (1992)	4.3	16	b
	Quehenberger et al. (1990)	CFT	3917	3.8	8	a,e
Bangladesh	Biswas et al. (1993)	IHA	200*	20	64	b
Brazil						
Amazon	Cavalcante et al. (2006)	MAT	80	37.5	25	a,f
Minas Gerais	Guimarães et al. (1992)	IFA	198	90.4	16	a,e
Paraíba	Azevedo et al. (2009)	IFA	130	36.2	50	a
Paraná	de Moura et al. (2007)	IFA	117	8.54	64	b
	Garcia et al. (1999)	IFA	267	24	64	a
	da Silva et al. (2008)	MAT	304	7.2	64	a,b
Rio Grande do Sul	Grünspan et al. (1995)	IHA	200*	18	64	a
São Paulo	Suaréz-Aranda et al. (2000)	ELISA	300	9.6	NS	a,f
	dos Santos et al. (2005)	MAT	286*	17	25	b,d
São Paulo and Pernambuco	Caporali et al. (2005)	MAT	759	1.32	64	a,f,c
Canada	Smith (1991)	MAT	1443†	9.4	40	a
	Gajadhar et al. (1998)	LAT	2800*	8.6	32	b,e
Ontario	Poljak et al. (2008)	ELISA	6048*	0.74	NS	a,c
Chile	Tamayo et al. (1990)	DT	1474	28.1	16	a,c,f
Costa Rica	Arias et al. (1994)	IFA	496	43.8	20	a,e
Czech Republic	Hejlíček and Literák (1993)	DT	2616	5.9	4	b,f
	Vostalová et al. (2000)	DT	259	0.4	4	b,f
	Sedlák and Bártová (2007)	IFA	20	20	64	
Germany	Fehlhaber et al. (2003)	ELISA	1005	20.5	NS	a,c,d
	Damriyasa et al. (2004)	ELISA	2041†	18.5	NS	a,e,f
	Damriyasa and Bauer (2005)	ELISA	1500†	9.3	NS	a
	de Buhr et al. (2008)	ELISA	4999†	*4.1	NS	a
Ghana	Arko-Mensah et al. (2000)	ELISA	641	40.6	NS	a,e,f
Indonesia	Inoue et al. (2001)	LAT	208	6.25	64	b
Italy	Genchi et al. (1991)	IFA	90	64.4	40	a,c,e
	Villari et al. (2009)	ELISA	3472	10.4	NS	a,c
Korea	Jeon et al. (1988)	ELISA	482	15.1	NS	a
Malaysia	Rajamanickam et al. (1990)	IHA	122	15.6	64	
	Chandrawathani et al. (2008)	IFA	100	0	200	
The Netherlands	Berends et al. (1991)	ELISA	23,348	2.1	NS	b
	van Knapen et al. (1995)	ELISA	994*	1.8	NS	b,e
		ELISA	1,009†	30.9	NS	b,e
	Meerburg et al. (2006)	ELISA	2796*	3	NS	b,c
	van der Giessen et al. (2007)	ELISA	265 €	0.38	NS	a,c
		ELISA	178**	5.62	NS	a,c
		ELISA	402‡	2.74	NS	a,c
	Kijlstra et al. (2004)	LAT	660‡	1.2	64	a,b
		LAT	635**	4.7	64	a,b
		LAT	621	0	64	a,b
	Kijlstra et al. (2008)	ELISA	406	10.9	NS	
Panama	Correa et al. (2008)	IFA	290 €	32.1	20	b
Peoples Republic of China	Cui et al. (1989)	ELISA	338	23.6	NS	f
		IHA	338	9.5	NS	f
	Lin et al. (1990)	IHA	816	10.4	64	
	Huang (1991)	IHA	525	20.19	64	
	Zou et al. (in press)	IHA	831	16.9	64	b
Peru	Suaréz-Aranda et al. (2000)	ELISA	96	32.3	NS	a,f
	Saavedra and Ortega (2004)	WB	137	27.7	NS	b,e

Table 1 (*Continued*)

Country	Reference	Test	No. exam	%Pos.	Cut-off titer	Notes
Poland	Bartoszcze et al. (1991)	ELISA	925	36.4	100	a
	Sroka et al. (2008)	MAT	106	26.4	40	b,f
Portugal	de Sousa et al. (2006)	MAT	333	15.6	20	b,d
Serbia	Klun et al. (2006)	MAT	605	28.9	25	c,e
Sweden	Ljungström et al. (1994)	MAT	60	50	40	a,f
	Lundén et al. (2002)	ELISA	695*	3.3	NS	b
		ELISA	110†	17.3	NS	b
Taiwan	Chang et al. (1990b)	LAT	3880	27.7	NS	e
	Fan et al. (2004)	LAT	111*	28.8	32	b
	Tsai et al. (2007)	LAT	395*	10.1	32	a,c
Uruguay	Freyre et al. (1991)	DT	601	70.2	16	e
USA						
Georgia	Saavedra and Ortega (2004)	WB	152	16.4	NS	b,e
Hawaii	Dubey et al. (1992)	MAT	509	48.5	25	a
Illinois	Weigel et al. (1995b)	MAT	5080†	20.8	25	a,e
		MAT	1885*	3.1	25	a,e
	Dubey et al. (1995b)	MAT	4252*	2.3	25	a
		MAT	2617†	15.1	25	a
Iowa	Smith et al. (1992)	MAT	273†	14.3	32	a
Maryland	Dubey et al. (2008)	ELISA	48**	25	NS	a
		MAT	48**	70.83	25	a,d
Massachusetts	Dubey et al. (2002)	MAT	55*	92.7	10	a,d
Mid west	Gebreyes et al. (2008)	ELISA	616*	4.1	NS	c
NAHMS[$]	Zimmerman et al. (1990)	ELISA	2029*	4.78	NS	a
		ELISA	587*	10.05	NS	
NAHMS	Patton et al. (1996)	MAT	3479†	20	32	a
	Hu et al. (1997)	MAT	3473	19	32	
NAHMS	Patton et al. (1998)	MAT	4712*	3.2	32	a
			3236†	15	32	a
NAHMS	Patton et al. (2000)	MAT	8086 †	6	32	a
			5720*	0.9	32	a
NAHMS	Hill et al. (2009)	ELISA	6,238	2.65	NS	a,f
Nationwide	Dubey et al. (1991a)	MAT	11229*	23	25	b
			613†	42	25	b
New England states	Gamble et al. (1999)	MAT	1897	47.4	25	a,c
North Carolina	Davies et al. (1998)	MAT	2238*	0.58	32	a
Tennessee	Assadi-Rad et al. (1995)	MAT	3841†	36	32	a,c
Vietnam	Huong and Dubey (2007)	MAT	587	27.2	25	a,b,e
Zimbabwe	Hove and Dubey (1999)	MAT	97	9.3	25	
	Hove et al. (2005)	IFA	*238	19.7	50	f
		IFA	†70	35.1	50	f

Type or category of pigs. (*) Market, (†) sows, (‡) organic, (**) free-range, (€) intensive, ($) NAHMS: National Animal Health Monitoring Systems. Serologic tests. CFT: complement fixation test; DT: dye test; ELISA: enzyme linked immuno-sorbent assay; IFA: indirect fluorescent antibody; IHA: indirect hemagglutination antibody; LAT: latex agglutination test; MAT: modified agglutination test; WB: western blot. Parameters: a, farms; b, abattoir; c, risk factors; d, isolation; e, age; f, serorogic test comparison; NS, not stated.

antibiotic treatment in their lifetime. If the pig requires more extensive treatment, it loses its status as an organic pig and must be sold as a (cheaper) regular pig. Because of the bovine spongiform encephalitis (BSE) crisis in the EU, farmers there are forbidden to feed their pigs products of "animal" origin.

In addition, *T. gondii* antibodies were detected by ELISA in 42 of 807 (5.2%) meat juice samples from pigs in Sweden (Lundén et al., 2002), and 12 (0.5%) of 2094 in the USA (Dubey et al., 2005).

2.2. Serological tests comparison on sera from naturally infected pigs

Results in many serological surveys in Table 1 are not comparable because of differences in techniques and cut-off values used. Only a few studies used more than one test for these serological surveys. Tamayo et al. (1990) reported 28.1% and 30.1% seropositivity in dye test and IHA, respectively in 1474 pig sera (1:16 dilution) from Chile. Chang et al. (1990b) found antibodies to *T. gondii* in 27.5% by LAT (cut-off 1:32) and 47.1% by ELISA of 3880 pigs from Taiwan. Ljungström et al. (1994) found 50% seroprevalence using 1:40 serum dilution both in the IFA and MAT on 60 pig sera from Sweden. Hirvelä-Koski (1990) reported a better correlation between ELISA and IFA than ELISA and IHA on a select group of 100 pig sera from Finland; the 100 sera were selected out of 1849 slaughtered pigs on the basis of ELISA testing. Cavalcante et al. (2006) found *T. gondii* antibodies in 37.5% of 80 pigs from the Amazon region in Brazil by MAT (titer 1:25) and in 43.7% of the same pigs by IFA (titer 1:64).

Damriyasa et al. (2004) in Germany found 18.5% *T. gondii* seropositivity for by ELISA and 16.5% by IFA (cut-off 1:16).

Sroka et al. (2008) assayed 106 sera from pigs from Poland by MAT, LAT, IFA, and an in-house ELISA and found 28%, 23%, 36%, and 35% seropositivity, respectively. The cut-off value for MAT was 1:40, but they did not state cut-off values for other tests. The MAT and LAT are commercially available. Most authors have used in-house ELISAs and thus results are difficult to compare; standardization of cut-off values and antigens are needed. There are no commercial ELISA kits for the detection of *T. gondii* antibodies in pigs. Villari et al. (2009) used the ELISA kit marketed by Institute Pourquier, France for the detection of *T. gondii* in pigs. This Pourquier kit can be used for several mammalian species because its conjugate is a G-protein able to bind with high affinity the Fc portion of various classes of immunoglobulins from numerous species (Villari et al., 2009).

2.3. Demonstration of T. gondii in tissues of naturally infected pigs

Viable *T. gondii* organisms were isolated from tissues of pigs collected from abattoirs or farms (Table 2). The success of isolation varied, in part due to bioassay procedures. Bioassay in cats increased the isolation rate because much larger volumes of tissue (500 g or more) can be bioassayed in a cat as compared with mice. Selection of tissues by screening of donor pigs for *T. gondii* antibodies before bioassay increased the efficiency of isolation versus bioassay of all tissues, irrespective of antibody status in the donor pig.

Among the studies listed in Table 2, Dias et al. (2005) isolated *T. gondii* from 13 of 149 pork sausages in Brazil. The sausages were made fresh with an unknown contact time with salt (<2 h) and 50 g samples were digested in pepsin and bioassayed in mice. Viable *T. gondii* was isolated from one sample; for the 12 other samples mice inoculated with sausage digests developed *T. gondii* antibodies in IFA (cut-off 1:16), but viable parasites were not found.

Serological or parasitological surveys based on abattoir samples do not provide a true assessment of risk to humans, because post slaughter treatment of meat (storage, treatments with salts) can affect the viability of tissue cysts. For example, nearly half of the pork in retail meat in the USA is injected with salts and water (Dubey et al., 2005). Some of the salt treatments (labelled as "enhanced" meat) kill *T. gondii* tissue cysts (Hill et al., 2004). Dubey et al. (2005) conducted an extensive survey of retail pork sold in the USA for the prevalence of viable *T. gondii*. Based on a sampling plan designed to estimate prevalence within 0.5%, 2094 unfrozen pork samples were obtained from 28 metropolitan statistical areas. Each sample consisted of 1 kg of boneless pork. In total, 100 g of each of 2094 samples was bioassayed in cats; *T. gondii* was isolated from seven of 2094 samples.

In addition to individual samples bioassayed (Table 2), Wikerhauser et al. (1988) isolated viable *T. gondii* from three of 10 pools of diaphragms from adult pigs in Yugoslavia; there were 10 to14 diaphragms in each pool. Freyre et al. (1991) isolated *T. gondii* from pools of diaphragms of pigs in Uruguay. Gajadhar et al. (1998) did not isolate viable *T. gondii* from the hearts of any of 2800 pigs in Canada; however, 8.6% of pigs were seropositive. The mouse bioassay was used by Wikerhauser et al. (1988), Freyre et al. (1991), and Gajadhar et al. (1998).

In addition to demonstration of viable *T. gondii* in pork listed in the foregoing discussion, *T. gondii* DNA was found in pork samples from Brazil and Japan. da Silva et al. (2005) reported *T. gondii* DNA by PCR in 19 of 70 (27.1%) sausages from 55 establishments from São Paulo, Brazil, and Belfort-Neto et al. (2007) found *T. gondii* DNA in 34% of 50 diaphragms and 66% of 50 tongues from pigs from abattoirs from Erechim, Brazil. Zakimi et al. (2006) reported that 57 of 101 lymph node samples from pigs from an abattoir on Okinawa Island, Japan contained *T.*

Table 2
Isolation of viable *T. gondii* from tissues of naturally infected pigs.

Country	Type	No. exam.	No. positive (%)	Tissues biossayed	Isolate designation	Genetic data	Reference
Argentina	Market	109	14 (12.8)[a]	Diaphragm	No	No	Omata et al. (1994)
Austria	Market	253	1 (0.4)[a]	Brain, heart, diaphragm	No	No	Edelhofer (1994)
Brazil	Market	28[b]	7 (25)	Heart, brain, tongue	Yes	Yes	dos Santos et al. (2005)
	Market	12	6 (50)[a]	Brain	No	No	Frazão-Teixeira et al. (2006)
	Sausages	149	13 (8.7)[a] (see text)	Sausage	No	No	Dias et al. (2005)
Czech Republic	Market	2447	29 (1.1)[a]	Brain, diaphragm	No	No	Hejlíček and Literák (1993)
Portugal	Market	37[b]	15 (40.5)	Heart, brain	TgPiPr1–15	Yes	de Sousa et al. (2006)
USA	Market	38[b]	14 (36.8)[a]	Heart	TgPgUs1–14	Yes	Dubey et al. (2008); Velmurugan et al. (2009)
	Sows	1000	170 (17)[a]	Heart	TgPg Us15–99	Yes	Dubey et al. (1995a) and Velmurugan et al. (2009)
	Market	300	29 (9.6)[c]	Heart	TgPgUs100–128	Yes	Velmurugan et al. (2009)
	Market	55	51 (92.7)[c]	Heart, tongue	TgPg Us129–179	Yes	Dubey et al. (2002), Lehmann et al. (2003), and Velmurugan et al. (2009)
	Retail meat	2094	7 (0.3)[c]	Loin	TgPgUs180–182	Yes	Dubey et al. (2005)

NS: not stated.
[a] Isolation in mice.
[b] Seropositive.
[c] Isolation in cats.

gondii DNA; this is an unusually high prevalence of *T. gondii* in a lymphoid tissue from chronically infected pigs.

2.4. Genotyping of T. gondii isolates from pigs

Pigs are considered the most important meat source of *T. gondii* for humans in the USA and efforts are being made to genetically compare *T. gondii* isolates from pork with those from humans to get an understanding of transmission. Until recently, *T. gondii* was considered clonal with little genetic diversity. Most of the *T. gondii* isolates could be divided into three genotypes I, II, III based on limited genetic markers and strains from North America and Europe (Howe and Sibley, 1995). Type I strains are uniformly lethal for mice, irrespective of the dose whereas Type II and III are less pathogenic for mice. However, there are no correlates of the *T. gondii* genetic type in higher animals. Recent studies using isolates from many countries and using recently developed markers indicated much higher genetic diversity than previously recognized (Lehmann et al., 2006; Dubey and Su, 2009). In my laboratory, a total of 182 *T. gondii* isolates (designated TgPgUs1-182) from domestic pigs from various sources in the USA were genotyped (Velmurugan et al., 2009; Dubey and Su, 2009) using 10 PCR-RFLP markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico). Nine genotypes (#1–9) were recognized from the 182 *T. gondii* isolates. Most isolates (56%, 102) were clonal Type II (genotypes #1 and #2) while 27% (49) were clonal Type III (genotype #3) strains. Genotype #4 had Type II alleles with the exception of Type I alleles at loci Apico and L358. Eight isolates (genotype #5) from Iowa had a combination of alleles I, II, and III at different loci and all of them were from sows; whether these isolates were recombinant strains is not known. Eight of the nine genotypes from pigs were previously reported in different animal species and geographical regions, suggesting that there are no species-specific strains of *T. gondii.* The remaining six isolates were divided into genotypes #6–9 and had a combination of different alleles. In conclusion, along with the predominance of clonal Type II and III strains, a few diverse, previously unrecognized *T. gondii* lineages were found circulating in domestic pigs.

Using the SAG2 PCR-RFLP marker dos Santos et al. (2005) found that of the seven *T. gondii* isolates from pigs from Brazil, two isolates were Type I and five were Type III. de Sousa et al. (2006) genotyped 15 *T. gondii* isolates from pigs from Portugal; 11 were Type II, and four Type III, using SAG2 2 and 6 microsatellite markers.

da Silva et al. (2005) and Belfort-Neto et al. (2007) directly amplified *T. gondii* DNA from pork samples from Brazil. Using nested PCR and SAG2, da Silva et al. (2005) reported that of 19 *T. gondii* DNA samples from sausages, 14 were Type I and five were Type III. However, recent studies using 10 RFLP markers and sequencing have revealed that unlike other countries, *T. gondii* isolates from a variety of animals in Brazil are not clonal and Type II is rare (Dubey and Su, 2009). Belfort-Neto et al. (2007) reported that of the DNA samples directly amplified from pork, one was Type I at SAG2, and Type III at BTUB, SAG3, and GRA 6.

Table 3
Antibody titer (MAT) and isolation of viable *T. gondii* from hearts of 1000 sows by bioassays in mice and cats[a].

MAT titer	No. of sows	No. of isolates	%Positive
<20	778	29	3.7
20	35	13	37.1
40	21	8	38.1
80	50	30	60
200	32	24	75
400 or higher	29	22	75.8

[a] From Dubey et al. (1995c).

2.5. Validation of serological tests for T. gondii

At the request of the US National Pork Producers Council, a validation study for methods to detect *T. gondii* was performed on hearts from 1000 sows from a sausage plant in Osceola, Iowa in 1989–1992 (Dubey et al., 1995c). Homogenates of 100 g from heart tissue were digested in pepsin, and inoculated into 10 mice per heart. A total of 10,000 mice were inoculated with pig heart digests. Additionally, pig sera were screened with MAT and 183 hearts were bioassayed in cats. Sera were retrospectively tested for *T. gondii* antibodies using IHA, LAT, and ELISA; the IHA and LAT were performed using commercial kits and the ELISA was based on a crude *T. gondii* tachyzoites lysate (Dubey et al., 1995b). In total, *T. gondii* was isolated from 170 pigs (Table 2), 50 by bioassay in mice, 58 by bioassay in mice and cats, and 62 by bioassay in cats only. Isolation of *T. gondii* generally increased with antibody titer (Table 3). It is noteworthy however, that 29 of 170 isolates were from pigs considered to be seronegative (MAT <1:20); 17 of these pigs were found to have MAT titer of 1:10 on further testing (Dubey et al., 1995c). None of the 108 isolates killed mice on initial passage. Judging from the mouse infectivity data, it appears that only a few *T. gondii* was in the inocula. Of the 10 mice inoculated with each of 108 infected pig hearts, the percent frequency of *T. gondii* positive mice was 32.4 (1/10 mouse positive), 12 (2/10 mice positive), 14.8 (3/10 mice), 7.4 (4/10 mice), 1.8 (5/10 mice), 3.7 (6/10 mice), 9.2 (7/10 mice), 0.9 (8/10 mice), 6.4 (9/10 mice), and 11.1 (10/10 mice positive). In other words, only in 12 of 108 cases all 10 inoculated mice developed *T. gondii* infection. The sensitivity and specificity respectively of different serological tests were calculated to be 82.9% and 90.29% for MAT, 29.4% and 98.3% for IHA, 45.9% and 96.9% for LAT, 72.9%, and 85.9% for ELISA based on the isolation of the viable *T. gondii* from tissues of pigs (Dubey et al., 1995b).

The data on serology and isolation presented in Table 3 are from a large number of naturally exposed pigs, irrespective of the serology. To my knowledge, there are no such data available from other studies in any animal species infected with *T. gondii*. Data from other reports on pigs are summarized in Table 4. Omata et al. (1994) isolated *T. gondii* from three of 40 pigs with IFA titers of 1:16 or lower but they did not state how many pigs were lower than 1:16.

Gamble et al. (2005) further evaluated MAT and ELISA on sera from 274 market age pigs naturally exposed to *T. gondii*; 70 pigs were bioassay positive and 204 were

Table 4
Antibody titers and isolation of *T. gondii* from naturally infected pigs.

Reference	Omata et al. (1994)	dos Santos et al. (2005)	de Sousa et al. (2006)	Dubey et al. (2002)	Dubey et al. (2008)
Serologic test	IFA	MAT	MAT	MAT	MAT
All pigs or only seropositive	All	Seropositive	Seropositive	All	Seropositive
No. of pigs bioassayed	109	28	37	55	38
No. of *T. gondii* isolates	14	7	15	51	14
Antibody titer					
<10				1/5	1
16	3/40[a]				
20			1	3/3	1
40			4	2/2	2
64	5/57				
80			10		3
100 or more		1		38/38	7
200		4			
1000 or higher	5/12	2			

[a] No. of *T. gondii* positive/no. of pigs.

bioassay negative by tests performed in cats. The ELISA used in this study was based on whole tachyzoites coated plates in a kit marketed by Safe-Path Laboratoies (Gamble et al., 2005). Good correlation was obtained between ELISA and MAT; of the 70 bioassay-positive pigs, 60 were positive by MAT at a cut-off of 1:25, and 67 were positive at a cut-off of 1:10; 62 were positive by serum ELISA. Of 204 bioassay negative pigs, 193 were negative in MAT (cut-off 1:25); giving a specificity of 94.6% at a cut-off of 1:25; 98.0% were negative at cut-off of 1:10. Use of tissue fluids in the ELISA yielded poor results; only 40 of the 70 bioassay-positive animals gave positive values.

Similar results were obtained with another study with 55 market pigs from a farm in Massachusetts (Dubey et al., 2002). In this study, attempts were made to isolate *T. gondii* from 55 pigs, irrespective of antibody status. Hearts (30 pigs, batch 1) or hearts and tongue (25 pigs, batch 2) were fed to cats; 51 of the 55 cats shed *T. gondii* oocysts. In the first batch, all 30 pigs had MAT antibodies of 1:100 or higher and all cats fed these tissues shed oocysts. In the second batch, 21 of 25 cats fed pig tissues shed oocysts; the four bioassay negative pigs had MAT titers of <1:10 (Table 4). It is of interest that two bioassay positive pigs had MAT titers of 1:10 and 1:20 and these were also seropositive by western blot (Dubey et al., 2002).

Overall, whole tachyzoite ELISA and MAT gave good results, but some infected pigs were seronegative. Until now, recombinant ELISA, including p30 antigen has not performed better than an ELISA using a crude lysate (Gamble et al., 2005).

2.6. Clinical toxoplasmosis

Severe clinical toxoplasmosis in pigs is considered rare and reports prior to 1988 were summarized by Dubey (1986) and Dubey and Beattie (1988). Since 1988, there have been reports of clinical toxoplasmosis in weaned pigs (Weissenböck and Dubey, 1993; Okamoto et al., 1989; Liao et al., 2006) and in neonatal pigs (Kumagai et al., 1988; Haritani et al., 1988; Chang et al., 1990b; Giraldi et al., 1996; Thiptara et al., 2006).

Okamoto et al. (1989) reported simultaneous outbreaks of acute toxoplasmosis on five farms in Japan, perhaps associated with feed contaminated with oocysts. On a farm in Austria, 13 of 80 feeder pigs became ill (Weissenböck and Dubey, 1993). Clinical signs included anorexia, fever, dyspnea, limb weakness, and seven died. Three pigs that died and two surviving pigs were examined histopathologically. *T. gondii* was found in lesions and the diagnosis was confirmed immunohistochemically. All seven pigs had high dye test titers (1:65536). Epidemiological investigation suggested that pigs probably became infected by ingesting food contaminated with oocysts (Weissenböck and Dubey, 1993). Liao et al. (2006) reported clinical toxoplasmosis in two sows from People's Republic of China. Both sows died. *T. gondii* tachyzoites were seen microscopically in smear made from bronchopulmonary lymph node of sow 1 but not sow no. 2. However, *T. gondii* was isolated from tissues of both sows by bioassays in mice, and the diagnosis was confirmed by PCR.

Kumagai et al. (1988) reported neonatal toxoplasmosis in pigs. Seven piglets were born to one of seven sows on a farm in Japan, four of these piglets were stillborn. Three of the seven piglets had gait abnormalities and died before they were 15 days old. On necropsy, the piglet had evidence of encephalitis, pneumonitis, and lymph node necrosis. The diagnosis was confirmed immunohistochemically, and by finding antibodies to *T. gondii* (Kumagai et al., 1988).

Haritani et al. (1988) reported congenital toxoplasmosis in four stillborn and one live born piglet in Japan. Encephalitis and *T. gondii* was identified in all five piglets. Four of these animals also had pneumonia, and two had hepatic necrosis.

Chang et al. (1990b) diagnosed toxoplasmosis abortion in 20 of 120 swine on eight farms in Taiwan during June 1987 to July 1988. Degenerative changes associated with tachyzoites were found in placenta and fetal tissues, but they did not provide quantitative data.

Neonatal toxoplasmosis in pigs in Brazil was reported by Giraldi et al. (1996). They found *T. gondii* in tissues of two aborted fetuses, six stillborn, and 10 neonatal piglets; in histological sections of brains from 15 piglets, hearts of 13 piglets, lungs of 12 piglets, livers of 11 piglets, retinas of 10 piglets and spleens of five piglets.

Table 5
Risk assessment studies on toxoplasmosis in pig.

Country	No. of pigs	No. of farms	Method	Main findings	Reference
Germany	117 farms, mixed	Farms selected out of Federal State of Hesse	Personal interviews	Piglet production versus pedigree breeding identified as risk	Damriyasa et al. (2004)
Italy	Slaughter house, data,	274 farms	Slaughter house data, and interview with farmers	Source of water, altitude, number of pigs on the farm	Villari et al. (2009)
The Netherlands	2796 100–400 pigs, mixed type	41 organic 3 organic	Questionnaire Rodent control	Cats and goat whey Rodent control dramatically reduced prevalence	Meerburg et al. (2006) Kijlstra et al. (2008)
USA	Mixed Mixed Sows Mixed 6238	120 mixed 47 mixed 107 select farms 1 farm 185 facilities, 16 states	Questionnaire Farm visits, all wildlife tested Questionnaire, 107 of 303 farms replied Genotyping of *T. gondii* isolates from pigs and wildlife NAHMS	Cat access, rodents Juvenile cats, house mice Out door housing and size of the farm Infection originated from pigs Disposal of pigs on the farm affected seroprevalence	Weigel et al. (1995a) Assadi-Rad et al. (1995) Lehmann et al. (2003) Hill et al. (2009)

A seropositive (LAT titer 1:64) sow in Thailand gave birth to three stillborn and six live piglets (Thiptara et al., 2006). The piglets had signs of dyspnea and bloody diarrhea. One live and one stillborn piglet were examined at necropsy. Toxoplasmosis was diagnosed in both piglets based on finding of tachyzoites in impression smears of the lungs. Antibodies (LAT titer 1:64 or higher) were found in three of three suckling piglets, five of six growing piglets, one boar, and one of four sows on the farm.

Venturini et al. (1999) found a low rate of congenital *T. gondii* infection in stillborn piglets in Argentina; IFAT antibodies (titer 1:20) were detected in 15 of 738 fetal fluids, and 10 of these were also positive by MAT (1:25 titer).

Recently, Kim et al. (2009) reported an outbreak of clinical toxoplasmosis in adult sows in a larger herd in Jeju Island, Korea. Affected sows were febrile, anorectic, had neurological signs, and few aborted. *T. gondii* was found histologically in tissues of four adult sows and five aborted littermate piglets.

2.7. Risk assessment

Risk assessment studies are summarized in Table 5. Management of pigs varies a great deal within a region and within countries. An extensive study of risk factors for transmission of *T. gondii* on pig farms in Illinois was undertaken by Weigel et al. (1995b). In the first phase, at least 30 sows on each of 123 farms were tested for *T. gondii* antibodies and telephone interviews were conducted with producers to assess risk factors; higher seroprevalence in sows was associated with cat access to sows. In the second part of this study, all the risk factors were assessed on 47 farms by actual visits to the farms and examination of domestic and wildlife sources of *T. gondii* infection. The presence of seropositive juvenile cats and higher seroprevalence of *T. gondii* infection in house mice were the two main risk factors for *T. gondii* infection in pigs on these 47 farms (Weigel et al., 1995b). Vaccination of cats on eight of these farms with a live T-263 vaccine to prevent oocyst shedding apparently reduced seroprevalence of *T. gondii* infection in pigs, although the experiment lacked proper controls (Mateus-Pinilla et al., 1999, 2002).

Meerburg et al. (2006) and Kijlstra et al. (2008) investigated farm-epidemiology of toxoplasmosis on organic swine farms in The Netherlands. In the first study, 2796 pigs from 41 farms were tested for *T. gondii* antibodies at slaughter; *T. gondii* was found in 3% of pigs (Meerburg et al., 2006). Based on analysis of responses to a questionnaire sent to farmers, the number of cats on the farm, and feeding of goat whey were positively associated with seroprevalence in pigs (Meerburg et al., 2006). In a more recent study (Kijlstra et al., 2008), three organic farms with a rodent problem were studied further. During a four-month period, 215 rodents were trapped on these farms; *T. gondii* DNA was detected in heart or brain tissue of 11 of 71 rodents from farm 1, one of five rodents from farm 2, and none of 25 rodents from farm 3. A total of 406 pigs from these three farms (121 farm 1, 201 from farm 2, and 84 from farm 3) were tested for *T. gondii* antibodies at slaughter. Overall, the seropositivity in these three farms decreased from 10.9% to 3.3% at the end of rodent control study. However, the prevalence increased after the rodent control had stopped. These studies indicate a positive role of rodent control in the prevention of *T. gondii* in pigs on organic farms in The Netherlands (Kijlstra et al., 2008). The source of *T. gondii* infection (oocysts, tissue cysts) is at issue because organic pigs can be raised *T. gondii*-free. In Germany, Schulzig and Fehlhaber (2005) reported up to 15.2% seroprevalence in pigs raised conventionally, but zero prevalence in pigs on organic farms.

Pigs can be infected from a variety of sources, including wild and domestic animals in the immediate proximity of pig barns. Lehmann et al. (2003) used genetic and ecological methods to study transmission on a pig farm in the USA. They isolated viable *T. gondii* from pigs and other domestic and wild animals on the farm and genetically compared isolates from pigs and from animals at different locations on the farm. The results indicated

Table 6
Experimental toxoplasmosis in pigs fed oocysts.

Stage, strain, dose	No. and age	Period days	Main observations	Reference
GT1, oocysts, 1000	4, 4–5 month	125–875	Persistence of viable	Dubey (1988)
Me49, 1000	4, 4–5 month	103–759	*T. gondii* in tissues	
TC2, 1000	4, 4–5 month	103–822		
TS2, 1000	4, 4–5 month	103–782		
Unknown, 1000 or 10,000	3, 6-week old	9 weeks	Irradiation of meat, 1 pig died day 8 p.i.	Wikerhauser et al. (1989)
GT1 or PT1, 1000	17, pregnant	32–92	Congenital toxoplasmosis	Dubey and Urban (1990) and Dubey et al. (1990)
Ten strains mixed, 10,000–100,000	8, 3–5 month	64	Protection, biology	Dubey et al. (1991b)
GT1, 100,000	4	28–471	Persistence of viable *T. gondii* in tissues, protection	Lindsay et al. (1993)
GT1, 80,000	2	48	Persistence of viable *T. gondii* in tissues, protection	Pinckney et al. (1994)
VEG 10	13, 2–3 month	69–97	Infectivity dose	Dubey et al. (1996a, 1996b)
VEG 1	16, 2–3 month	22–99		
SSI, 1000	9, 8 weeks	84–102	Disease in general	Wingstrand et al. (1997) and
SSI, 10,000	10, 8 weeks			Lind et al. (1997)
VEG, 40,000	30, 7 weeks		Protection, biology	Garcia et al. (2005), Garcia et al. (2006a), Garcia et al. (2008) and Tsutsui et al. (2007)
AS-28, 50,000	8, 4 months	47	Diagnosis	Yai et al. (2003)

that the transmission of *T. gondii* was higher near the pig sties than in the surroundings in terms of strain composition and risk of infection. They concluded that *T. gondii* infection in pigs most likely originated in the pig barn and not from outside the barn.

Recently, Villari et al. (2009) found that origin of pigs (foreign versus born locally), age of the pigs, management, number of pigs on the farm, and source of water were the main factors for *T. gondii* seroprevalence in pigs in Sicily, Italy. Seroprevalence in market age pigs was 7% and the highest than in neighbouring countries. The altitude of the farm and the number of animals were also important risk factors; seroprevalence was lower on farms at higher altitudes (>200 meters) and on farms with less than 50 pigs on the farm.

Using the 2006 NAHMS data, Hill et al. (2009) found that rodent control and carcass disposal methods affected the seroprevalence in pigs on 185 swine production facilities in 16 states of the US. Burial or composting of dead pigs on the farm was a risk factor for increased seroprevalence as was the lack of professional rodent control services on the farm.

3. Experimental infections in domestic pigs

Studies have shown that pigs can be infected by inoculation of tachyzoites, and by ingestion of oocysts or tissue cysts (Dubey, 1986; Dubey and Beattie, 1988; Wingstrand et al., 1997). Data on pigs fed oocysts are summarized in Table 6. Certain aspects of these infections will be reviewed.

3.1. Clinical

Weaned pigs fed oocysts or tissue cysts generally developed weight loss, anorexia, fever, but generally recovered by three weeks p.i., irrespective of the *T. gondii* isolate.

3.2. Immunity

3.2.1. Humoral responses

Pigs inoculated with any infectious stage of *T. gondii* produce humoral antibodies detectable by various serological tests (Chang et al., 1990a; Dubey et al., 1994; Dubey et al., 1996b; Lin and Hung, 1996; Andrews et al., 1997; Lind et al., 1997; Jungersen et al., 1999; Singh, 2000; Gamble et al., 2000; Garcia et al., 2006a, 2006b; Jongert et al., 2008). Pigs inoculated intravenously with tachyzoites developed IgM by 8–10 days p.i. and IgG antibodies by 10–17 days p.i. (Lind et al., 1997; Jungersen et al., 1999). Pigs inoculated with *T. gondii* tachyzoites, tissue cysts, or oocysts developed specific antibodies detectable by four types of ELISAs and cross reactivity was found when sera of pigs infected with various pathogens (*Ascaris suum*, *Trichinella spiralis*, *Isospora suis*, *samonella* spp, *Yersnia* or *Actinobacillus* spp.) of pigs were tested, except *Sarcocystis miescheriana*; low cross reactivity was not found in sera from three of nine pigs infected with *S. miescheriana* (Lind et al., 1997). However, this cross reactivity between *T. gondii* and *S. miescheriana* was not found by Dubey (1997) using the MAT; perhaps related to the type of serologic test used. In MAT, whole *T. gondii* tachyzoites were used and the antibody reacted to surface antigens whereas in ELISA the parasite lysate was used and antibody reacted to internal antigens. Lin and Hung (1996) also did not detect cross reactivity between *T. gondii* antigens and other swine pathogens (*Actinobacillus pleuropneumoniae*, hog cholera virus, porcine reproductive and respiratory syndrome virus, pseudorabies virus, *Ascaris suum*) using the avidin–biotin ELISA, but they did not test for antibodies to *Sarcocystis* spp.

Naïve pigs fed even a few oocysts responded serologically, and specific response was verified by parasite isolation. *T. gondii* was isolated from tissues of 13 of 14 pigs fed 10 oocysts and 17 of 28 pigs fed one oocyst (Dubey et al., 1996a). Naïve pigs fed 1–10 oocysts developed

antibodies to *T. gondii* by three-week p.i. by MAT, but seroconversion as measured by IHA and LAT was delayed and titers remained low (Dubey et al., 1996b).

3.2.2. Protective immunity

Pigs develop good protective immunity as evidenced by recovery from infection with viable *T. gondii*. The cellular basis of this Th1-related cytokine response was characterized in pigs fed oocysts (Solano Aguilar et al., 2001; Dawson et al., 2005). However, pigs can develop fever, diarrhea, dyspnea, and can die depending on the dose and the age of the pig. The production of proinflammatory cytokines such as interferon-gamma during the acute phase is thought to be responsible for this pathogenic effect (Solano Aguilar et al., 2001). Limited studies using partially genetically defined miniature pigs fed oocysts indicated no effect of host MHC in mediation of protective immunity (Dubey et al., 1998).

Pigs inoculated with modified *T. gondii* strain (RH strain), mutant strain (Ts-4), or irradiated strains developed protective immunity following a challenge with an unaltered strain of *T. gondii*. The RH strain of *T. gondii*, originally isolated from a child in 1938, is one of the most pathogenic strains of the parasites for mice, but not for pigs. It has been passaged in mice since 1938 and most lines of this parasite do not produce tissue cysts in mice. After intramuscular inoculation of 100,000 RH tachyzoites, pigs develop a febrile response, and viable parasite can be recovered up to 14 days p.i. but not later (Dubey et al., 1991b; Dubey et al., 1994). Pigs developed good protective immunity in the absence of demonstrable viable parasites as evidenced by failure to recover viable *T. gondii* in five of eight pigs following a lethal challenge with oocysts; this immunity was not strain specific because pigs were challenged with oocysts of the 10 *T. gondii* isolates with all three genotypes (Dubey et al., 1991b). The Ts-4 is a temperature sensitive mutant of the RH strain, and it is non-pathogenic for mice. Nursing pigs inoculated intravenously with Ts-4 strain remained asymptomatic and had fewer tissue cysts in their tissues when challenged with oocysts of the GT-1 strain of *T. gondii* (Pinckney et al., 1994). The GT1 strain is a genetically Type 1 strain of *T. gondii* and is highly pathogenic for mice.

Pigs fed *T. gondii* oocysts irradiated with 0.4 kGy cesium developed protective immunity in the absence of demonstrable viable *T. gondii* and humoral antibodies (Dubey et al., 1998). *T. gondii* oocysts irradiated at 0.25 kGy cesium loose infectivity but protective antigens are not destroyed. Pigs vaccinated with irradiated oocysts remained clinically normal following a lethal oocyst challenge but developed tissue cysts; thus, reinfection with *T. gondii* was not prevented.

Attempts are being made to develop non-infectious vaccines for toxoplasmosis in pigs with some success. Garcia et al. (2005) reported that pigs vaccinated with crude rhoptry proteins developed partial protection based on reduction in tissue cyst numbers in pigs challenged with oocysts. Pigs vaccinated intradermally with dense granule-associated proteins (GRA1–GRA7) cocktail DNA vaccine developed antibodies and cellular immunity (Jongert et al., 2008).

3.3. Comparison of diagnostic methods

Yai et al. (2003), Hill et al. (2006), Garcia et al. (2006b, 2008), and Tsutsui et al. (2007) compared histopathlogy, bioassay, and PCR for the detection *T. gondii* bradyzoites and concluded that PCR and histopathology were insensitive for the detection of tissue cysts. Yai et al. (2003) compared PCR and bioassay to detect *T. gondii* in tissues of 8 pigs killed 47 days after feeding 50000 oocysts of the As-28 strain; *T. gondii* was detected in tissues of 5 pigs by PCR and by bioassay in mice from tissues of 4 pigs. Hill et al. (2006) compared the efficacy of serum serology, tissue extract serology, real time PCR, nested PCR, and direct PCR for the detection of *T. gondii* in pork using samples from 25 naturally infected pigs from a farm, 10 experimentally infected pigs, and 34 retail meat samples, and then ranked detection methods in the following descending order of sensitivity: serum ELISA (test sensitivity 100%), serum MAT (80.6%), tissue fluid ELISA (76.9%), real time PCR (20.5%), semi-nested PCR (12.8%), and direct PCR (0%). Neither ELISA nor MAT reliably detected antibodies in frozen and thawed muscle samples (Hill et al., 2006).

Garcia et al. (2006b) compared PCR, bioassay, and histopathology in 10 pigs fed *T. gondii* oocysts and killed 60 days later; *T. gondii* was detected in 55.1% of 98 muscle samples by bioassay in mice, in 16.6% of 150 muscle samples by PCR and in 0 samples by histopathology (Garcia et al., 2006b). These authors also found *T. gondii* DNA in three of 10 aqueous humour samples from eyes of these pigs (Garcia et al., 2008).

Tsutsui et al. (2007) compared distribution of *T. gondii* in commercial cuts of pork by bioassay and PCR in 10 pigs 59 days after feeding them oocysts; *T. gondii* was found in 67.5% by bioassays in mice and 22.5% of samples by PCR.

The differences in sensitivity of different diagnostic techniques compared by Yai et al. (2003), Hill et al. (2006), Garcia et al. (2006b, 2008), and Tsutsui et al. (2007) are probably due to the biology of *T. gondii* infection in pigs. The serological tests (MAT, ELISA) assayed antibodies directed against systemic dissemination of infection and the persistence of antibodies for a long time. The PCR methods detected parasite proteins in a very small amount (<1 g) of host tissue; the concentration of *T. gondii* in pigs is very low (one tissue cyst in 25–100 g of tissue). The histological method was the least sensitive because in a 5 μm histological section, less than 10 mg of host tissue is examined.

3.4. Congenital toxoplasmosis

A review of published data suggested that it is difficult to produce congenital toxoplasmosis consistently in swine (Dubey and Beattie, 1988). The reasons for failure and success are not well understood. The stage of *T. gondii*, route of inoculation, stage of gestation, and breed of sow may account for some of these variations. Some of these factors are reviewed.

Dubey and Urban (1990) fed 1000 oocysts to 17 sows at 32–92 days of gestation (Table 5). Six sows produced transplacentally infected fetuses; one aborted an infected fetus 17 days after ingestion of oocysts. Many fetuses were

Table 7
Seroprevalence of *T. gondii* in feral pigs.

Country	Reference	Test	No. exam.	%Pos.	Cut-off titer
Austria	Edelhofer et al. (1989)	IFA	364	17.9	16
	Edelhofer et al. (1996)	IFA	269	19.3	NS
Brazil	Fornazari et al. (2009)	MAT	306	4.5	16
Czech Republic	Hejlíček et al. (1997)	DT	124	15	4
	Bártová et al. (2006)	IFA	565	26.2	40
Germany	Lutz (1997)	IFA	130	25	80
Japan					
Kukamoto	Shiibashi et al. (2004)	LAT	90	4.4	64
Iriomote Island	Nogami et al. (1999)	LAT	108	5.6	64
Slovak Republic	Antolová et al. (2007)	ELISA	320	8.1	NS
Spain	Gauss et al. (2005)	MAT	517	38.4	25
	Ruiz-Fons et al. (2006)	MAT	91	363	25
USA					
Georgia	Dubey et al. (1997)	MAT	170	18.2	25
South Carolina	Diderrich et al. (1996)	MAT	257	34.2	32

DT: dye test; ELISA: enzyme linked immuno-sorbent assay; IFA: indirect fluorescent antibody; IHA: indirect hemagglutination antibody; LAT: latex agglutination test; MAT: modified agglutination test; NS: not stated.

mummified. Examination of sera before and after birth indicated that antibodies were not transmitted across the placenta and colostrum-derived antibodies disappeared by three months of age. Placentas and fetal tissues from two of these litters of pigs were selected for detailed histopathological examination (Dubey et al., 1990). Sow 1 was fed oocysts at 60 days of gestation and killed 49 days later; it had eight dead fetuses, of which one was mummified. Sow no. 2 was fed oocysts at 45 days of gestation and killed 62 days later (gestational term 120 days); it had 11 fetuses of which four were mummified, two were dead, and five were live.

Lesions and *T. gondii* were found in seven fetuses from sow 1 and three fetuses from sow 2. The predominant lesion was necrotizing placentitis, and numerous tachyzoites were observed in trophoblast cells lining the areolae of the placenta. Of the 10 fetuses with toxoplasmosis, lesions were present in the brains of eight, hearts of seven, spinal cords of six, lungs of four, skeletal muscle of four, livers of three, tongues of three, kidneys of one, and eye of one. An unusual finding was that tachyzoites were demonstrable histologically but not by bioassay, indicating that in some fetuses tachyzoites died with host cells. The myocarditis was characterized by multifocal necrotizing myocarditis, mineralization, and tachyzoites in myocytes (Dubey et al., 1990).

Using inbred minipigs, Jungersen et al. (2001) found that some *T. gondii* strains were more pathogenic than others. They inoculated 10 pregnant sows intravenously with 30,000 tachyzoites of five *T. gondii* strains (two per sow). Four sows inoculated with SVS P14, and SVS Fox 2 *T. gondii* strains aborted fetuses 10–11 days p.i.; abortion occurred presumably due to pyrexia in the dam. Generalized toxoplasmosis was found in three fetuses from two sows inoculated with SVS014 strain and fetuses from one of two sows inoculated with the SSI 119 strain; sows were killed 48 days p.i. The two sows inoculated with NED strain were killed 49 days p.i.; in one sow, fetuses were not infected, and in the second sow 1 fetus had localized infection in the allantochorion (Jungersen et al., 2001).

3.5. Persistence and distribution of *T. gondii* in tissues

Long time persistence was investigated in 16 pigs fed oocysts of one of the four *T. gondii* (GT1, Me49, TS2, TC2) strains (Dubey, 1988) *T. gondii* persisted as long as 875 days p.i.; *T. gondii* was recovered from tissues of 14 of 16 pigs: from brains of 12 of 16 pigs, hearts of 11 of 16 pigs, tongues of 10 of 16 pigs, and diaphragms of six of 16 pigs. The parasite was isolated from all commercial cuts of pork; of the seven pigs killed 350–875 days p.i. viable *T. gondii* was isolated from five infected pigs; from the shoulder picnic and ham of three, Boston butt, spare ribs and tenderloin of two and bacon and tail bone of one. It is noteworthy that *T. gondii* persisted in liver of a pig killed at 759 days p.i. and kidney of a pig killed 875 days p.i.

4. Toxoplasmosis in wild pigs

Seroprevalences are summarized in Table 7. Seroprevalence varied from 4–34%. Dubey et al. (1997) found a very low seroprevalence (0.9%, 11 of 1264) in pigs from Ossabaw Island, which is a remote island off the coast of Georgia. There is an absence of any type of cat on this island. The low prevalence was attributed to grain that was imported from the mainland USA for feeding pigs; it was hypothesized that the grain could have been contaminated with *T. gondii* oocysts or birds could have carried infection with them. The 11 sera found positive by MAT (titers 1: 20–640) were also seropositive by the dye test, and LAT, and 10 of 11 were also positive by the IHA. By comparison, seroprevalence in feral pigs on the main land was high; MAT antibodies were found in 31 (19.2%) of 170 feral pigs. The seroprevalence in pigs increased with age, indicating postnatal transmission of *T. gondii* in feral pigs.

Feral pigs in the USA are hunted for food, and the consumption of improperly cooked pork can be a source of human infection. However, I am not aware of any report on the isolation of *T. gondii* or clinical toxoplasmosis in feral pigs. However, three adult humans acquired toxoplasmosis after feasting on viscera of a wild pig in Korea resulting in loss of vision and blindness (Choi et al., 1997). In another episode, five of 11 persons developed acute toxoplasmosis following ingestion of raw pig liver in Korea (Choi et al., 1997).

5. Conclusion

Review of literature indicates that pigs can be raised in confinement *T. gondii*-free if there is adequate rodent control and contamination of feed and water with oocysts can be prevented. More research is needed to define conditions for controlling *T. gondii* infection in free-range or organically raised pigs. Studies indicate that pumping certain salts in pork to make it more tender or improve its shelf life kills *T. gondii*. Further research is needed to standardize conditions (curing, salting) for killing *T. gondii* in processed pork. There is a need to improve antigens for ELISA for the detection of *T. gondii* antibodies in pigs. Currently used molecular or histological methods are insensitive to detect *T. gondii* in pork because the density of this parasite in meat is low (one tissue cyst per 25 g or more).

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